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Soybean Protein and Oil Percentages Determined by Infrared Analysis¹

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SUMMARY

Protein and oil percentages in soybean (*Glycine max* (L.) Merrill) seed meal were determined by an infrared light reflectance instrument (IR) and compared with standards for protein analysis by Kjeldahl and oil analysis by the Soxhlet pet ether extraction method. Within the ranges for which the IR instrument is calibrated, it will do a satisfactory job.

The correlation coefficient between IR protein and Kjeldahl protein determinations of the soybean seed meal used in the calibration was 0.995, and the correlation coefficient between IR oil and Soxhlet pet ether extraction of oil was 0.983. Tests of independent soybean samples gave correlation coefficients between IR protein and Kjeldahl protein of 0.971 and between IR oil and Soxhlet pet ether extraction oil of 0.977.

INTRODUCTION

The official American Oil Chemist Society (AOCS) methods for protein and oil determinations are by Kjeldahl and Soxhlet pet ether extraction,³ respectively. However, the recent application of infrared spectral reflectance (IR) opens a new area in chemical grain analysis. Dr. K. H. Norris, Agricultural Research Service, Instrumentation Research Laboratory, Beltsville, Md., deserves much of the credit for the pioneering work

that is responsible for the spectral reflectance method.^{4 5 6 7 8}

An earlier report⁷ has described the use of an IR reflectance instrument for the use of protein and oil estimations made by the DICKEY-john Corporation of Auburn, Ill. In this publication, reported is the calibration of an infrared light reflectance instrument made by Neotec Instruments, Inc. of Rockville, Md.

MATERIALS AND METHODS

The infrared light reflectance instrument used for this study was a Neotec Infrared Quality Grain Analyzer Model 32-E-2000. The instrument is housed in a room that is maintained at 25°C with a relative humidity of 45 to 62 percent.

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³American Association of Cereal Chemists. AACC approved methods. (7th ed.) The Association, St. Paul, Minn. 1962.

⁴BEN-GERA, I. and K. H. NORRIS. DETERMINATION OF MOISTURE CONTENT IN SOYBEANS BY DIRECT SPECTROPHOTOMETRY. Israel J. Agr. Res. 18: 125-132. 1968.

⁵_____ and K. H. NORRIS. DIRECT SPECTROPHOTOMETRIC DETERMINATION OF FAT AND MOISTURE IN MEAT PRODUCTS. J. Food Science 33: 64-67. 1968.

⁶HART, J. R. and K. H. NORRIS. DIRECT SPECTROPHOTOMETRIC DETERMINATION OF MOISTURE CONTENT OF GRAIN AND SEEDS. 1963 International Symposium on Humidity and Moisture Proc. 4: 19-25. 1965.

⁷HYMOWITZ, T., J. W. DUDLEY, F. I. COLLINS and C. M. BROWN. ESTIMATION OF PROTEIN AND OIL CONCENTRATION IN CORN, SOYBEAN AND OAT SEED BY NEAR-INFRARED LIGHT REFLECTANCE. Crop. Sci. 14: 713-715. 1974.

⁸MASSIE, D. R. and K. H. NORRIS. SPECTRAL REFLECTANCE AND TRANSMITTANCE PROPERTIES OF GRAIN IN THE VISIBLE AND NEAR INFRARED. Trans. of ASAE 8: 598-600. 1965.

From each of 40 bulk samples (1800 gm each) of viable seed collected, approximately 100 gms of each sample were used in the calibration of the instrument. Nitrogen analysis was done by the Kjeldahl method,³ and the nitrogen values were multiplied by the factor 6.25 to give estimates of percent protein. Oil analysis was done by Soxhlet pet ether extraction.³ All analyses were duplicated and will be referred as standard laboratory values.

The IR instrument was calibrated to determine protein and oil percentages in the range of 5 to 9 percent moisture content of the meal. This was accomplished by dividing the 40 samples into 5 moisture groupings. The first group of seeds had their moisture content adjusted to between 4 and 5 percent; the second group between 5 and 6 percent; the third between 6 and 7 percent; the fourth between 7 and 8 percent; and the fifth between 8 and 9 percent.

When the moisture content of the seeds had to be raised, the whole seeds were placed in a Stults Scientific Seed Germinator at 25°C and 99.9 percent humidity. When the moisture content of the whole seeds had to be lowered, the whole seeds were placed in a Thelco oven at 27°. In either case, the whole seeds were checked at hourly intervals and the moisture content was determined by the official AOCS method.³ Approximately 20 grams of seeds were used in this step.

After the whole seeds had been adjusted to the proper moisture, 20 grams of seeds were ground in a Mitey-Mill (Stur-Dee Health Products, Co., Inc., Island Park, N.Y.) for 35 seconds. The meal was mixed thoroughly and placed to overflowing into the Neotec sample cup. The meal was leveled with a spatula and placed into the spring loaded

compressor and tightened. The entire cup was cleaned outside and then placed into the loader drawer and the drawer closed. Readings for protein, oil and moisture were read directly from the instrument. In the calibration steps these are termed C readings.

The 40 standard laboratory values for protein, oil and moisture, along with the respective 40 Neotec C readings were used in solving the following equations to obtain a least squares multiple regression.

$$\text{Protein \%} = K_0 + K_1C_1 + K_2C_2 + K_3C_3 \quad (1)$$

$$\text{Oil \%} = K_0 + K_1C_1 + K_2C_2 + K_3C_3 \quad (2)$$

$$\text{Moisture \%} = K_0 + K_1C_1 + K_2C_2 + K_3C_3 \quad (3)$$

K_0 is the intercept and K_1 , K_2 and K_3 are the regression coefficients associated with C_1 , C_2 and C_3 , respectively.

Included for calibration purposes were 180° rotation Neotec C readings for each of the 40 standard samples. Rotation of the cup 180° gives insurance that the machine is reading samples consistently and that the sample has been prepared properly.

After the K values are solved for each of the three constituents, the respective K values were placed in the instrument and the 40 samples were rerun. The average value for a constituent from all the samples from the standard laboratory values and from the values obtained from the IR instrument should be approximately the same. If they are not, the K_0 may be adjusted accordingly to obtain a better fit.

Duplicate Kjeldahl nitrogen and Soxhlet pet ether oil extractions were also carried out on 45 independent soybean samples and then the protein and oil percentages of the 45 independent samples were determined by the calibrated IR instrument.

RESULTS AND DISCUSSION

Figure 1 shows the relationship between the 40 standard laboratory protein percentages and the corresponding 40 calculated protein percentages (calculated from equation 1). The coefficient of determination (r^2) for the multiple regression equation was 0.986. The standard deviation (mean squared deviation from regression) was 0.8425.

Figure 2 shows the relationship between the 40 standard laboratory oil percentages and the corresponding 40 calculated oil percentages (cal-

culated from equation 2). The coefficient of determination (r^2) for the multiple regression equation was 0.972. The standard deviation (mean squared deviation from regression) was 0.6541.

The standard laboratory values for the 40 samples used in the calibration of the instrument ranged from 28.8 to 51.4 percent for protein and from 15.0 to 27.2 percent for oil. After initial readings were obtained from the instrument for the 40 samples, the K_0 readings for protein and oil were adjusted as mentioned in Materials and

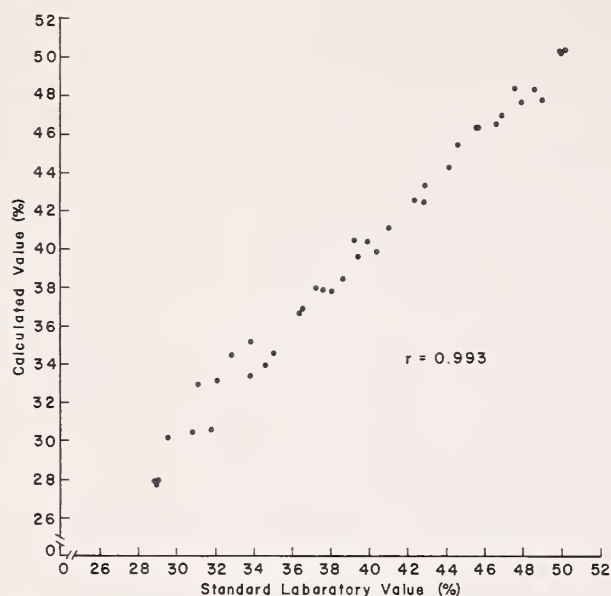


FIGURE 1.—Relationship between the 40 standard laboratory protein percentages and the corresponding 40 calculated protein percentages. (Calculated from equation 1.)

Methods. The 40 samples were then rerun and the correlation coefficient between the IR protein and Kjeldahl protein and between IR oil and Soxhlet oil were 0.995 and 0.983, respectively. The correlation coefficient between IR moisture and AOCS moisture was 0.970.

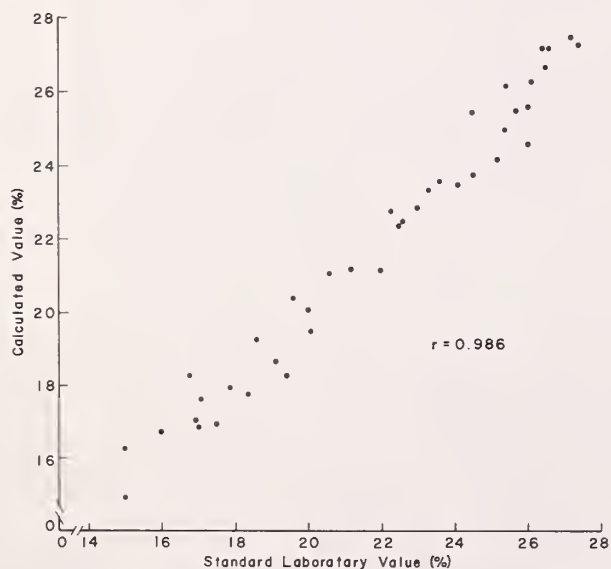


FIGURE 2.—Relationship between the 40 standard laboratory oil percentages and the corresponding 40 calculated oil percentages. (Calculated from equation 2.)

TABLE 1.—Standard laboratory values and infrared reflectance values for 45 independent soybean samples

	Protein		Oil	
	Kjeldahl	IR	Soxhlet	IR
	Percent	Percent	Percent	Percent
	32.0	32.0	25.8	25.4
	32.0	33.3	25.8	25.7
	32.5	32.0	25.8	25.3
	32.6	31.6	25.5	25.2
	32.9	33.2	26.5	26.3
	34.0	34.4	26.6	25.9
	34.9	36.7	23.6	22.5
	36.2	35.9	25.0	24.8
	36.3	36.1	22.2	22.7
	36.5	35.8	26.0	25.4
	36.6	36.7	21.8	20.6
	36.7	36.2	24.1	23.6
	36.7	36.6	24.2	23.9
	36.9	38.0	21.4	20.8
	37.5	37.7	25.4	25.9
	37.9	38.0	22.6	21.8
	38.3	37.7	22.8	21.4
	38.5	37.6	21.7	22.0
	38.6	38.9	23.9	22.5
	38.7	38.8	24.2	23.7
	38.8	37.6	24.2	24.5
	38.9	38.4	24.4	24.4
	39.4	39.4	23.1	23.4
	39.4	39.4	23.2	22.9
	39.4	39.6	23.6	22.6
	39.6	39.9	22.3	23.3
	39.6	39.2	23.3	22.4
	39.6	39.7	22.3	21.8
	39.9	39.7	22.8	22.6
	40.0	40.0	21.4	20.5
	40.4	40.3	20.1	19.7
	40.5	40.7	21.4	22.0
	40.5	40.2	20.1	19.8
	42.1	41.8	24.7	24.3
	42.9	42.5	24.2	23.9
	43.1	42.8	19.8	18.5
	44.1	43.2	23.0	22.8
	45.3	45.7	17.0	16.7
	46.2	46.5	19.6	19.4
	47.2	46.1	16.4	15.3
	48.2	47.3	17.8	17.3
	48.4	47.0	19.9	19.4
	48.8	48.0	15.2	15.7
	50.1	49.4	16.1	16.1
	50.2	48.6	16.5	16.6
Mean	39.8	39.6	22.4	22.0

Correlation coefficient of IR protein to Kjeldahl protein 0.971.

Correlation coefficient of IR oil to Soxhlet oil 0.977.

IR protein was then compared with Kjeldahl protein and IR oil with Soxhlet oil of 45 independent soybean samples. Table 1 gives the Kjeldahl protein and Soxhlet oil values along with the corresponding IR values. Protein in the 45 samples ranged from 32.0 to 50.2 percent and the oil from 15.2 to 26.6. The correlation coefficient between IR protein and Kjeldahl protein and between IR oil and Soxhlet oil were 0.971 and 0.977 respectively.

The IR instrument provides another means of estimating protein and oil in soybean seeds when the seeds have been ground into meal. After calibration of the instrument, the operation of the instrument is relatively simple and straightfor-

ward. We have found, however, that grinding of the sample is a critical factor. This fact is obvious because the instrument's accuracy depends on light reflectance off of the sample's surface.

Once the operator outlines a standard working procedure, within the range of protein and oil for which the instrument is calibrated, the instrument will do a satisfactory job. When in operation, the instrument automatically displays to the operator the percent protein, percent oil, and percent moisture of the sample in the cup. Though only having calibrated the instrument for soybean meal, the instrument could also be used for other grains if properly calibrated. One convenient feature of the IR instrument is that at no time do the samples have to be weighed.

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